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Autologous platelet-rich-plasma injection and platelet-rich fibrin glue interposition for treatment of anal fistula resistant to surgery

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ABSTRACT

Aim: The current study purposed to evaluate the autologous platelet-rich plasma (PRP), and platelet-rich fibrin glue (PRFG) effect on the treatment of complex, and recalcitrant anal fistula (AF) which was not cured by several surgeries.

Background: AF has remained one of difficult challenges for centuries. Surgery is the common treatment method for it, but the risk of fecal incontinence and recurrence is still a distressing complication for patients and surgeons. New procedures were published in the scientific literature, each with advantages and disadvantages. According to reports, an effective therapy option is the autologous fibrin glue that is rich in platelets.

Methods: Autologous PRP and PRFG were prepared from 10 patients' own blood. The surgeon curetted the tract of anal fistula for the deepithelialisation till hemorrhage occurred; PRP was injected around the fistula into the tissue, and PRFG was interpositioned in the tract. Age, number of previous surgeries, complications, number of PRP and PRFG administrations, and duration of halting the discharge were among the information gathered. Patients were followed up between 10 months to 84 months after treatment.

Results: No complications were observed during and after the injection. During the period of follow-up, AF leakage was stopped for 6 patients, but not for 4 patients.

Conclusion: Since autologous PRP injection, and PRFG interposition is a safe, effective, and minimally invasive procedure for resistant AF to surgeries; it can be used, along with surgery to increase the healing rate of complex anal fistula.

Keywords: Anal fistula, Platelet rich plasma, Fibrin glue, Healing.

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Introduction

An anal fistula (AF) is a tunnel connecting the anal canal or rectum (internal opening) with the skin (external opening) around the anus. Patients who have a perianal abscess have symptoms include discomfort, trouble sitting, and pus or blood discharge. Simple and complicated anal fistulas are the two types that exist. A complex fistula is difficult to manage, has a higher risk of recurrence rates, and poses a greater threat to continence after surgery. AF was associated with significant morbidity, which is a devastating condition with profound effects on both the physical and psychological health of the patient. The leakage of anal liquid leaves the patient with a persistent odor resulting in serious consequences like isolation from society in terms of shame, rejection by a spouse, loss of status and dignity, and economic problems (1).

Approximately 20,000 to 25,000 newly confirmed cases of AF are reported in the USA each year, and the incidence of AF is 1.69 cases per 10,000 individuals in the UK (2).

Surgery (the advancement flap) is the gold standard therapy for AF. The purpose of surgery is to eliminate sepsis, encourage tract healing, and protect the internal and external anal sphincters' structural integrity. The most feared complications of procedures are the risk of fecal incontinence (10-35%), and recurrence (10-60%). Moreover, different types of surgical interventions are presented with variable success rates, ranging from 24% to 100%. Also, it is reported that post-operative incontinence rates could be as high as 35% (3).

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The other reported available options of AF treatment (which there is controversy about reproducing results) are: 1) surgical ligation of intersphincteric fistula tract (LIFT) with 76.5% mean success rate, 0 incontinence and 5.5% postoperative complication rate with a mean follow-up of 10 months, 2) Anal fistula plug with 24% to 88% success rate with a mean follow-up of 8 months, 3) Fibrin glue with 14% to 74%success with a mean follow-up of 4 months, 4) Fistula laser closure with the overall success of 81% with 7.4 months of follow-up, 5) Video-assisted anal fistula treatment with an overall success rate of 73.5% within 2-3 month of follow-up, 6) Adipose-derived stem cells solution was injected into the tract and into the walls of the fistula which overall success rate of 57% within 12 months of follow-up (1).

There are several studies that suggest platelets and fibrin glue may be used to close an AF. Here, we provide data on the first 10 AF patients enrolled in broader clinical research that evaluated the effectiveness of platelet-rich plasma (PRP) and platelet-rich fibroblast growth factor (PRFG) in the treatment of AF.

Methods

Patients

In this pilot study, ten patients with a complex high anal fistula which confirmed by the clinical exam and MRI, which did not respond to several surgical interventions were included. These patients did not accept the further surgical procedure. The etiology of AF in 9 patients was unknown and in 1 case was trauma. Table 1 lists the patient characteristics, operations, length of leaking time, PRP-FG applications, time till leakage stops following PRP-FG application, and follow-up period.

The study was conducted based on the principles of Declaration of Helsinki 1996 version, and Good Clinical Practice standards. The study protocol, informed-consent form, and other study-related documents were reviewed and approved by the Human Research Ethics Committee of Mashhad University of Medical Sciences (ethical code: 941101). All patients were able to read, understand, and be willing to sign the informed consent of the study. The inclusion criterion: The occurrence of atrial fibrillation (AF), despite at least two surgical treatments; the duration of AF exceeding six months; the absence of uncontrolled diabetes; the absence of pregnancy; the absence of a history of radiation therapy; the absence of any drugs being used to treat AF at the present time; the absence of any physical or mental condition.

Autologous PRP & PRFG preparation

A homemade procedure explained in detail in our pervious study (4). The platelets and FG were prepared based on standard procedures. Sixty milliliter peripheral blood was taken, and PRP was prepared by first centrifugation at 2000 × g for 2 min (for red blood cells sedimentation), and then second centrifugation at 4000 × g for 8 min (for platelets sedimentation). The supernatant plasma was separated and 4 ml platelet-rich plasma (PRP) was left. Using either the cryoprecipitate technique or the ethanol precipitation method, two biochemical processes were used to separate the plasma into the fibrinogen concentrate. In cryoprecipitate method, following a -70 ºC freeze and a 4 ºC thaw, plasma was centrifuged at 6500 × g for 5 minutes. The supernatant plasma was removed to a final volume of 2 ml. In the ethanol precipitation method, absolute ethanol at 0°C was added to the plasma (10% v/v), and fibrinogen was collected by centrifugation at 6500 × g for 15 min and supernatant plasma was removed to a final volume of 2 ml. 2 ml concentrated fibrinogen mixed with 2 ml platelets [final volume 4 ml: platelet-rich fibrinogen plasma (PRFP)]. One milliliter of thrombin was prepared from removed plasma by adding 10% calcium gluconate.

PRP injection and PRFG interposition to AF tract

One gram of Cephazolin was intravenously given 1 hour before the operation. Under general anesthesia, and in lithotomy position the exact location of fistula was determined by a metallic malleable probe inserted into the fistula via an external orifice. The tract fistula was cleaned with betadine 10% before PRP and PRFP were applied, and deepithelialization was carried out all around the fistula until a bleed developed. Then, 2 ml of PRP was injected around the fistula into the tissue (the penetration depth in injection was 5-6 mm), and 4 ml PRFP was mixed with 1 m thrombin and interpositioned into the tract. The operation time was about 20-30 minutes, the clot formation usually takes about 8 minutes, but the anesthesia was extended to 20 minutes to make sure a complete clot in the place of fistula. On the first day after the operation, the patient was complete bed rest and was discharged 1 day after the operation. An antibiotic was prescribed for 6 days. Following the last PRP-FG treatment, patients had follow-

up visits for a period of time ranging from 10 to 84 months. The patient was considered to be healed when the anal fistula leaking stopped and lasted for at least five months.

Results

There was no morbidity during and after the procedure. The mean age of patients was 39.83±8.17 (years). During the period of follow-up, AF leakage was stopped for 6 patients who considered themselves clinically cured. For 4 patients, leakage did not stop. None of the patients had fecal incontinence. The characterizations of patients are presented in Table 1.

Discussion

This study showed that PRP and PRFG could be applied as a non-invasive, safe, and effective treatment option, along with surgical intervention for patients with AF who did not respond to several surgical interventions. Long-term follow-up of 10 patients showed that 6 patients were completely cured and 4 patients did not.

It is reported that failure to anal treatment was not affected by age and sex, nor by smoking and obesity (5). There is evidence that epithelialization of the fistula tract (6) contributes to treatment failure. Consequently, this factor may explain why four patients did not respond to treatment. The diversity of approaches suggested for the treatment of complex AF indicates the fact that no procedure was shown to be fully satisfactory. These methods are surgery (the advancement flap, ligation of intersphincteric fistula tract), seton, filling therapy, etc. with controversial results (7).

Fistulotomy is used for the treatment of simple fistula with a healing rate of 90% and a low risk of incontinence. But since there is an unacceptably high risk of incontinence, it cannot be utilized for complicated fistula. Most patients choose for sphincter-preserving procedures despite the possibility of less favorable outcomes because lowering the risk of incontinence is crucially more important than having a greater healing rate (8). In the 1990s, the first study for the treatment of AF reported using fibrin glue (9). It is reported the cure rates of fibrin glue varied widely, from 14 to 86 %, with no provoking incontinence. During the same decade, PRP began to be used in surgery as a regenerative tissue factor (10).

As a filling therapy; fibrin glue, PRP, autologous cartilage, fat, autologous micro-fragmented adipose tissue, acellular dermal matrix, and allogeneic bone marrow-derived mesenchymal stromal cells were used to plug anal fistula (1).

Allogenic fibrin glue or autologous fibrin glue was used, along with or without PRP as less aggressive therapy during the last decade with a cure rate of 25% to 60% of cases without impairing continence, but with a high recurrence rate (11).

De La Portilla et al. demonstrated in a prospective double-blind randomized experiment (12) that the therapeutic results of two procedures, autologous PRP and autologous fibrin, were equal and that there were no adverse events. However, the recurrence rates of both procedures were significant, at 33.3 and 31.3%, respectively. In a multicenter study, Lara et al. reported the closing of complex AF in 66.6% of patients by curetting the fistula tract, sealing it with autologous platelet-rich fibrin, and closing the internal orifice (13).

In a clinical trial, Pérez Lara et al. applied autologous platelet-rich fibrin for AF outpatients and they found the same success rate compared to AF patients with surgical intervention (curettage and

Table 1. The characteristics of patients

Patient	Sex	Age	Number of operations	Number of PRP-FG application	Duration of healing*	Healed	Follow-up period**
1	Male	39	13	1	10	yes	84
2	Male	55	5	3	30	yes	60
3	Male	41	3	2	21	yes	36
4	Female	29	2	2	12	yes	24
5	Male	31	3	1	15	yes	24
6	Male	42	4	1	19	yes	36
7	Male	34	2	2	-	no	24
8	Female	28	3	1	-	no	12
9	Male	29	2	2	-	no	24
10	Male	36	3	2	-	no	12

*Duration of healing according to day; ** Follow-up period according to months.

sealing using a cylindrical-curette kit). They came to the conclusion that the outpatient treatment of AF was completely safe and a very affordable method. The outcomes were quite good and resembled those of surgically inserting a plug using conventional curettage. They mentioned that this approach should be considered a valid initial treatment for AF, before surgical treatment. This would prevent many complications which lead to considerable financial savings for the health system (14).

In a pilot study in the treatment of AF, Hagen et al. reported that autologous platelet-rich plasma as an adjunct to the staged mucosal advancement flap is a promising treatment modality with a high healing rate (15).

In AF, deepithelialisation and fibrous tissue removal around the fistula (less than 5 mm diameter around the fistula tract) were performed by debridement till hemorrhage was seen (which fistula turn into an acute wound), and a clot was formed there, but in the most cases, the fistula was not spontaneously healed (7). The reasons, which can be attributed to this fact, are contamination, infection, and inflammation of the tissue tract and may be inadequate blood supply at the area of repair.

AF closure is related to using platelets and FG together which are the main contributing factors in regenerative tissue. The optimum healing of a wound requires a well-orchestrated integration of the complex biological, and molecular events of cell migration, proliferation, extracellular matrix deposition, and remodeling, which growth factors, scaffold, and cells play an essential role in initiating and continuing these events. Consequently, the success and efficacy of fistula repair are contingent upon the provision of a robust scaffold and optimal local concentration of regenerative growth factors (16). As authors explained in previous experience of using PRP-FG for the treatment of vesicovaginal fistula, FG is a topical biological adhesive, which mimics the final stages of coagulation, where in thrombin splits off fibrinopeptide A and B from the fibrinogen chain to form a monomer, which polymerizes to form a fibrin clot at the site of application. A physiological clot is roughly ten times weaker than this one. In addition to acting as a crucial temporary extracellular matrix, this clot actively draws in cells to cause fibrin-mediated reactions as cell adhesion, migration, proliferation, and tubule formation (17). Fibrin clot promotes the occlusion of AF by providing a provisional matrix, and promoting the local proliferation, ingrowth of fibroblasts and collagen synthesis and subsequent replacement by connective tissue, new blood vessel formation, an influx of immune cells in a paracrine fashion and preventing fibrosis. Furthermore, fibrin clot was used for the delivery of growth factors to promote wound healing (18). The rationale beyond using PRP is its documented effect on wound healing, and the optimum concentration of platelets for wound healing is 4-5-fold times to concentration of platelets in the blood. The bioactive factors are in α-granules, and the dense granules. In tissue regeneration, the wound heals through the 3 phases inflammation, proliferation, and remodeling. These bioactive factors are active during each of these phases. The α-granules contain growth factors and cytokines, such as transforming growth factor–β (TGF-β), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I, IGF-II), fibroblast growth factor (FGF), epidermal growth factor, vascular endothelial growth factor (VEGF), and endothelial cell growth factor. These cytokines and growth factors are crucial for angiogenesis, chemotaxis, cell differentiation, and proliferation. Serotonin, histamine, dopamine, calcium, and adenosine are all present in the dense granules. These non–growth factors have fundamental effects on the biological aspects of tissue repair. In sum, the bioactive factors play a central role in the healing processes by modulating the recruitment, duplication, activation, and differentiation of different cell types in wound healing (19). Thus, PRP has antimicrobial activity against Escherichia coli, and Staphylococcus aureus, including methicillin-resistant staphylococcus aureus, candida albicans, and cryptococcus neoformans (20).

The use of autologous FG rather than allogenic FG, which lowers costs and avoids blood-borne infections that may be transferred through the commercially available FG, is the extra benefit of this therapy in this research. The limitation of our study is low sample size (a pilot study) which additional clinical trials should be run in multiple centers in order to substantiate its efficacy to increase the success rate of incurable AF.

Conclusion

In conclusion, in recalcitrant AF resistant to surgical treatment, and prone to anal incontinence, the

application of PRP- FG, along with surgical intervention gives a better chance for healing of this incurable AF. This additional treatment is completely innocuous, and non-invasive.

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Conflict of interests

The authors indicate no potential conflicts of interest.

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